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**Segregation analysis of overweight body condition in
an experimental cat population**

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Segregation analysis of overweight body condition in an experimental cat population

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Abstract

The goal of this study was to analyze the mode of inheritance of an overweight body condition in an experimental cat population. The cat population consisted of 95 cats out of which 81 cats could be clearly classified into lean or overweight using the body condition score system according to Laflamme. The lean or overweight classification was then used for segregation analyses. Complex segregation analyses were employed to test for the significance of one environmental and four genetic models (general, mixed inheritance, major gene, and polygene). The general genetic model fit the data significantly better than the environmental model ($P \leq 0.0013$). Among all other models employed, the major gene model explained the segregation of the overweight phenotype best. This is the first study in which a genetic component could be shown to be responsible for the development of overweight in cats.

Keyword: genetics, feline, overweight, BCS, inheritance

Introduction

Overweight and obesity are emerging health problems in the human society. In the year 2000 the WHO declared obesity as one of the greatest health problems of the western countries. In 2005 1.6 billion adults were overweight ($\text{BMI} \geq 25\text{kg/m}^2$), 400 millions were obese ($\text{BMI} \geq 30\text{kg/m}^2$) and the WHO predicts an increase in the next few years (www.who.int). Overweight and obesity are major problems not only in humans but also in companion animals as they represent two of the most common nutritional disorders in pets (Hawthorne et al. 2000; Prah et al. 2007). In cats, the prevalence of overweight ranges from 6 to 52 % (Anderson 1973; Colliard et al. 2008, Colliard et al. 2009). Multiple diseases are associated with overweight and obesity in cats, particularly diabetes mellitus type 2, lameness and dermatosis, which reduce life quality and can even lead to early death (Lund et al. 2005; Scarlett and Donoghue 1998). Risk factors like castration, indoor housing and access to highly palatable food lead to increased food intake and physical inactivity, resulting in a positive energy balance and finally in overweight (Colliard et al. 2008; Kienzle and Bergler 2006).

Besides these environmental factors the genetic background also plays an important role in the development of overweight. Family, twin and adoption studies in humans have given strong evidence that overweight is highly heritable and that 40 – 70 % of the variation in body mass index (BMI) of humans is due to genetic influence (Atwood et al. 2002; Maes et al. 1997). As a result of intensive investigations in mice and humans, mutations in different genes have been found to be responsible for the development of monogenic forms of obesity; however, monogenic forms of overweight are relatively rare and do not explain the rising problem (Clément et al. 1998; Farooqi and O'Rahilly 2004; Montague et al. 1997; Tartaglia et al. 1995; Vaisse et al. 2000; Zhang et al. 1994).

In theory, the development of an overweight phenotype is a multifactorial trait, characterised by a complex interaction of different genes and the environment (Comuzzie 1998; Li and Loos 2008; Loos and Bouchard 2003). As a result of genome-wide studies, multiple loci with strong associations to obesity have already been defined and in the coming years more are sure to emerge (Dina et al. 2007; Frayling et al. 2007; Scuteri et al. 2007; Meyre et al. 2009; Thorleifsson et al. 2009; Willer et al. 2009).

Similarly to the findings in humans, it is likely that overweight in cats is influenced by genetic factors. The goal of our study was to establish a plausible mode of inheritance for overweight in our experimental cat population.

Materials and Methods

Animals

A multi-generational feline pedigree segregating for the overweight phenotype was collected from the feline colony of the Institute of Animal Nutrition of the Vetsuisse-Faculty, University of Zurich. Cats were housed in groups of four to six cats, depending on enclosure size and maintained according to the animal welfare legislation of Switzerland. Cats were fed ad libitum with commercial dry food. At least two feeding places were available in each group.

The cat colony is a five-generation pedigree of mixed breed domestic cats and is composed of 95 intact individuals including 52 male and 38 female animals (Figure 1). The remaining 5 cats were stillborn and of unknown sex. The parent material consists of 48 cats (24 males and 24 females) aged 12 to 36 months. These animals were classified using the body condition score (BCS) according to the 9 point system of Laflamme (Laflamme 1997). A BCS <6 was considered lean and a BCS ≥ 6 was considered overweight. Based on the classification 14 experimental matings were performed resulting in a total of 47 kittens (28 males, 14 females, 5 stillborn). Classification into lean and overweight was performed using the same criteria as described above. For the segregation analyses the BCS estimated at the age of eight months was used to classify the kittens.

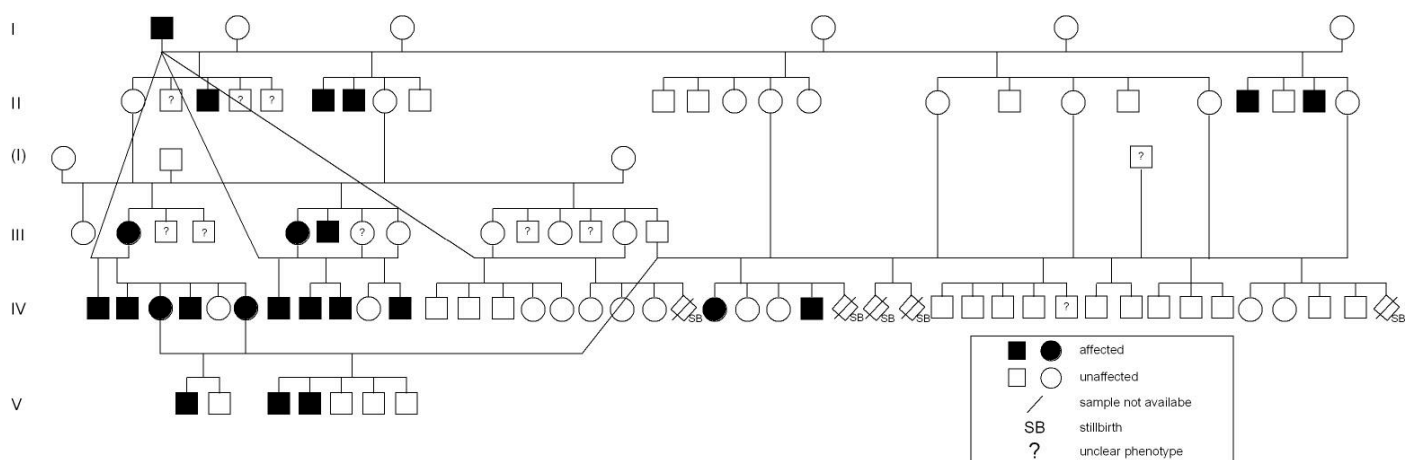


Fig. 1: Pedigree of the cat population. Animals were classified based on Body Condition Scores. Animals indicated with a question mark showed an unclear phenotype.

Segregation analyses

To elucidate a possible genetic background for obesity, complex segregation analyses were carried out comparing the ability of different models to explain the data.

As we assumed that the disease status is related to an underlying continuous variable and to a single threshold, we analysed our phenotype as a binary condition (lean, overweight). Likelihoods were computed for each model with the Pedigree Analysis Package (PAP) (Hasstedt 1994) and maximized with the program NPSOL (Gill et al. 1986). A maximum of seven parameters was estimated: allele frequency (1 parameter), transmission probabilities for the three genotypes of a biallelic major gene (3 parameters), dominance effect (1 parameter), displacement (1 parameter) and heritability (1 parameter). By dropping different parameters from the estimates five different models (environmental, general, major gene, polygenic, mixed inheritance) could be evaluated. In a first step a general genetic model including all seven parameters to also allow for a non-Mendelian inheritance of a major gene was compared to an environmental model where the major gene and the polygenic components were canceled by keeping the transmission probabilities equal to the allele frequency and the heritability equal to zero. In a next step, the environmental model could be rejected, the general genetic model is compared to a mixed inheritance model which is the same as the general genetic model with the exception that the transmission probabilities are set to be Mendelian. If the mixed inheritance model turns out best a major gene model and a polygenic model are compared to the mixed inheritance model. The major gene model is the same as the mixed inheritance model but it keeps the heritability at zero, whereas the polygenic model only estimates the heritability by setting the allele frequency to one. The $-2 \ln$ likelihoods of the models follow a Chi-square distribution and for the comparison of the models the degrees of freedom equal the difference in the number of parameters estimated. In addition to the 95 pedigree members a dummy sire had to be introduced for two full sibs with unknown sire. As no data were available to estimate a prevalence for overweight, prevalence's ranging from 0.20 to 0.40 in steps of 0.05 were used which covers the range of estimates based on more than 200 cats (Colliard et al. 2008; Lund et al. 2005; Scarlett et al. 1994).

Results and Discussion

A multi-generation pedigree consisting of 95 intact cats, segregating for an overweight phenotype, was analysed. The inbreeding coefficient assessed with the Pedigree Viewer 6.2 (<http://metz.une.edu.au/~bkinghor/pedigree.htm>) ranged between 0 % and 12.5 % and is similar or less to what has been seen in other populations (Calboli et al. 2008; Wiggins et al. 1997).

The parental material consisted of 48 cats of which 10 males and 21 females showed a normal body condition and were classified as lean. The remaining seven males and two females showed an overweight body condition and were classified as overweight.

The phenotype of eight cats (7 males and 1 female) could not be estimated as they were released to other institutions before this study started. Based on these phenotypes 14 experimental matings were designed, resulting in a total of 42 kittens born alive. Based on the BCS estimated at the age of eight months 16 male and 11 female kittens were considered lean, whereas 11 male and 3 female kittens were considered overweight. One male kitten died before reaching the age of 8 months and could not be phenotyped.

Body condition scoring using the 9 point system according to Laflamme is a subjective, semiquantitative method of evaluating body condition; however, it did yield reproducible results. Haering et al. (2011b) demonstrated that BCS, feline body mass index (FBMI) and dual energy x-ray absorptiometry (DEXA) measurement results are all related to each other and could be used as a tool to phenotype growing cats in general. Nevertheless, his results also pointed out, that the FBMI is not a reliable tool for growing cats and that DEXA measurements from various studies are difficult to compare.

The offspring were classified earlier (8 months) than the parental cats (12 – 36 months) and one may argue that the BCS should have been determined around the same age; however, once a cat has reached a stable body weight the body composition does not significantly change. When determining the BCS in kittens less than 1

year of age, one has to consider that the abdominal contour cannot be taken as criteria for kittens < 6 months as all these kittens have a pendulous abdomen (Lund et al. 1999). In all our offspring the BCS estimation was repeated when they reached 12 months of age and the scores remained unchanged from the 8 month estimation (Häring et al. 2011b).

A total of 81 cats from our experimental population had a clear distinct phenotype and were used for the subsequent segregation analysis. The results of the segregation analyses are shown in Table 1 and suggest strong evidence for an autosomal inheritance of a major gene. The general genetic model explained our data significantly better than the environmental model ($P \leq 0.0013$) regardless of different prevalences. No significant difference could be estimated between the general genetic model and the mixed inheritance model ($P > 0.802$). Consequently, the mixed inheritance model can be rated higher than the general genetic model as less estimated parameters are needed to explain the data. The mixed inheritance model was always better than the polygenic model ($P < 0.03$). No significant differences could be estimated between the major gene model and the mixed inheritance model; therefore, the major gene model was rated higher as less estimated parameters are needed to explain the data.

Tab. 1: Comparison of the -2 ln likelihoods of the five genetic models. Likelihoods for the different models were calculated under the assumption of different prevalences for overweight cats.

Model	Prevalence of overweight in cats				
	0.20	0.25	0.30	0.35	0.40
general	77.133	77.888	78.869	80.040	81.383
environmental	99.919	97.141	96.757	98.263	101.410
mixed inheritance	77.194	78.147	79.347	80.759	82.377
major gene	77.194	78.147	79.347	80.759	82.377
polygenic	82.162	83.077	84.173	85.428	86.837

Analysis of the estimated parameters for the major gene model revealed that with an increasing prevalence the frequency of the deleterious allele increased. The estimated allele frequency for the major gene ranged between 0.48 and 0.67 whereas the dominance effect was consistently zero (Table 2). The penetrance of the over-

weight phenotype was almost constant with values between 0.87 and 0.89. Computation of the displacement was not consistent between different runs, but it was always in the area of six (data not shown). The estimated parameters confirmed that an autosomal recessive major gene with incomplete penetrance is likely to be responsible for the observed phenotypic difference.

Tab. 2 : Estimated for the parameters of the major gene model. All parameters were calculated for different prevalences.

Prevalence	Frequency Allele a	Dominance	Penetrance of BCS		
			AA	Aa	aa
0.20	0.480	0.000	0.000	0.000	0.868
0.25	0.535	0.000	0.000	0.000	0.872
0.30	0.584	0.000	0.000	0.000	0.879
0.35	0.629	0.000	0.000	0.000	0.884
0.40	0.669	0.000	0.000	0.000	0.893

Although the population size is rather small, we believe that even with the small sample size evidence for a genetic background of the observed phenotype can be given. The favored two models did not change when different prevalences for overweight were used, indicating that our number of animals is sufficient to make a significant conclusion.

The major gene model was ranged higher as less estimated parameters are needed. Although the analysis favored a major gene model, we cannot totally exclude the mixed inheritance model. Likelihoods were identical in the major gene model and the mixed inheritance model; however, the major gene model was favored because less parameters for modeling had to be estimated. This might change in a larger sample size, but would also be in concordance with the findings in other species. Both genetic models can be taken into consideration as multiple loci responsible for an overweight phenotype have previously been identified in humans (Thorleifsson et al., 2009; Willer et al., 2009). The fact that we could not demonstrate the presence of a polygenic component could be due to the small family size but does not imply that no additional major or minor gene could play a role in the phenotype overweight in cats.

Further mating will be performed to confirm results and to strengthen our findings. In a second step it would be interesting to use molecular genetic approaches to characterize genes or genome regions involved in the development of overweight.

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